Br. J. Pharmac. (1974), 52, 349-358

EFFECTS OF IMIPRAMINE AND DESIPRAMINE ON RESPONSES OF SINGLE CORTICAL NEURONES TO NORADRENALINE AND 5-HYDROXYTRYPTAMINE

C.M. BRADSHAW, M.H.T. ROBERTS[1] & E. SZABADI
Department of Psychiatry, University of Edinburgh, Morningside Park, Edinburgh EH10 5HF

1 The technique of microelectrophoresis was used in order to study the effects of imipramine and desipramine on single neurones in the somatosensory cortex of the cat, anaesthetized with halothane.

2 Imipramine and desipramine, when applied for a brief period, did not affect the firing rate of the vast majority of the neurones tested.

3 Both potentiation and antagonism of excitatory responses to noradrenaline could be observed after a brief application of either of the antidepressants. Four drug-interaction patterns could be distinguished: potentiation of immediate onset; potentiation reaching its maximum after a delay; antagonism followed by potentiation; antagonism followed by recovery.

4 When different doses of the same antidepressant were applied, it was found that the drug-interaction patterns were related to the dose of antidepressant applied, a lower dose causing potentiation, and a higher dose antagonism of the response.

5 Both potentiation and antagonism of depressant responses to noradrenaline could be observed.

6 Both excitatory and depressant responses to 5-hydroxytryptamine were modified by imipramine and desipramine: a smaller dose of the antidepressant potentiated, and a higher dose antagonized the responses.

7 Excitatory responses to glutamate were not affected by imipramine and desipramine.

Introduction

It is generally believed that the tricyclic antidepressant drugs exert their antidepressant effects by blocking the (re)uptake of noradrenaline (NA) and/or 5-hydroxytryptamine (5-HT) into monoamine-containing nerve terminals, and thereby potentiating the pharmacological actions of the monoamines on post-synaptic receptor sites (Schildkraut, 1965).

This hypothesis is based on observations made in the peripheral nervous system and in different peripheral pharmacological test systems. Tricyclic antidepressants block the uptake of NA into sympathetically innervated tissues (Hertting, Axelrod & Whitby, 1961; Iversen, 1965), and they block the uptake of 5-HT into blood platelets (Todrick & Tait, 1969). These drugs are also able to potentiate the responses of adrenergically innervated tissues to exogenously applied NA (Sigg, Soffer & Gyermek, 1963; Sturman, 1970; McCulloch & Story, 1972) and 5-HT (Gyermek & Possemato, 1960; Sigg *et al.*, 1963), and to sympathetic nerve stimulation (Sigg *et al.*, 1963). It has been suggested that the potentiation of responses to NA is due to the blockade of uptake into nerve terminals (Hertting *et al.*, 1961; Iversen, 1965; Schildkraut, 1965). As imipramine and desipramine block the uptake of NA and 5-HT into brain tissue as well (Ross & Renyi, 1967; 1969), it has been assumed that the tricyclic antidepressants should potentiate the pharmacological effects of monoamines in the brain (Schildkraut, 1965; Davis, 1970).

We used the technique of microelectrophoresis in order to investigate how responses of single cortical neurones to the monoamines can be modified by tricyclic antidepressant drugs. Some of these results have been communicated to the British Pharmacological Society (Bradshaw, Roberts & Szabadi, 1971; Bradshaw, Roberts & Szabadi, 1973a). It has been reported by other workers that responses of brain stem neurones to

[1] Present address: Department of Physiology, University College of Wales, Cardiff CF1 1XL.

NA can be potentiated by imipramine (Avanzino, Ermirio & Zummo, 1971), and that responses of cerebellar Purkinje cells to NA can be potentiated by desipramine (Hoffer, Siggins & Bloom, 1971). There have been no reports, however, concerning the interaction between tricyclic antidepressants and 5-HT.

In this paper we describe how both excitatory and depressant responses of single cortical neurones to NA and 5-HT can be modified by imipramine and desipramine. We have found that the tricyclic antidepressants have a dual effect on responses to NA and 5-HT: lower doses potentiate, and higher doses antagonize the response.

Methods

Cats of either sex (2.0 to 3.5 kg) were used. Anaesthesia was induced by the intravenous injection of thiopentone and was maintained with halothane (0.5-1.2%) delivered from a temperature and flow-rate compensated vapouriser (Fluotec Mk III, Cyprane Ltd). Arterial blood pressure, ECG, EEG and CO_2 (%) at the level of the bifurcation of the trachea were monitored continuously throughout the experiment. The flow rate of oxygen required to maintain a constant inflation of the inspiratory reservoir bag was taken as a measure of the respiratory minute volume. In most experiments the animals respired spontaneously. Artificial ventilation was used if the spontaneous respiration became irregular, if the respiratory minute volume was markedly reduced, or if the end-tidal CO_2 exceeded 5%. A continuous infusion of a dextrose/saline solution (5% dextrose, 0.9% w/v NaCl solution) was administered at a rate of 4 ml/h, and the bladder was continuously drained through an indwelling catheter. Rectal temperature was maintained between 37° and 38°C with a heating pad controlled by a thermosensitive rectal probe.

Our technique for the preparation of an area of the anterior or posterior sigmoid gyrus has been described previously (Bradshaw & Szabadi, 1972).

Five-barrelled glass micropipettes were constructed and filled as described by Bradshaw, Roberts & Szabadi (1973b). The drug solutions used in these experiments were noradrenaline bitartrate (0.2 M, pH 3.0-3.5), 5-hydroxytryptamine bimaleate (0.2 M, pH 3.5), sodium glutamate (0.2 M, pH adjusted to 8.5 by the addition of 0.1 M NaOH), imipramine hydrochloride (0.2 M, pH 7.4), desmethylimipramine hydrochloride (0.15 M, pH 7.5).

The techniques used for recording action potentials, and for the electrophoretic application of drugs, are described by Roberts & Straughan (1967). A cumulative record of the total number of action potentials was obtained via a Grass UI-1 unit integrator.

All the neurones studied were spontaneously active. All the drugs were applied by microelectrophoresis. Repeated responses to the monoamines (or glutamate) were compared before and after a brief application of the antidepressant. Our measure of the dose of the antidepressant was the electrophoretic charge passed (intensity of electrophoretic current x time of passage of current). The sizes of excitatory responses to the agonists were expressed as the total number of spikes generated in response to each application of an agonist (total spike number). The total spike number was calculated by measuring the total spikes produced between the onset of the drug application and the recovery of the base-line firing rate, and subtracting the number of spikes emitted during an equivalent period before the drug had been applied (Bradshaw, Szabadi & Roberts, 1973). In the case of depressant responses, the total number of spikes generated between the onset of the drug application and the recovery of the base-line firing rate was subtracted from the number of spikes emitted during an equivalent control period before the drug had been applied, and the figure obtained was used as a measure of the size of the depressant response. The intervals between drug applications were kept constant as far as possible. During these intervals a retaining current of 25 nA was passed.

Results

Direct effect on neuronal firing

The direct effect of imipramine on the firing rate was studied on 137 cells; the effect of desipramine was investigated on 148 cells. The dose of antidepressant applied was 25-100 nA passed for 20-60 seconds. The effects observed are summarized in Table 1. It is apparent that the vast majority of the cells were not directly affected by the antidepressants. The excitation or depression observed was always of a temporary nature, and the original base-line firing rate recovered within a minute after the application of the antidepressant had been terminated. On occasions, a reduction in spike amplitude was observed; such cells were not used for drug-interaction studies.

On 29 cells, a longer, continuous application of the antidepressants was also tested (5-50 nA passed for up to 20 minutes). This continuous application of the antidepressant resulted in a cumulative effect on firing rate: first the firing rate was gradually reduced, and later a decrease in spike amplitude developed. This progressively

developing effect on spike amplitude could be observed even when very low ejecting currents were passed for a longer period. Therefore, a shorter application of the antidepressants was used for the drug-interaction studies described below.

Effect on responses to noradrenaline

Excitatory responses. Drug-interaction studies were successfully completed on 70 neurones responding with a clear increase in firing rate to NA. Cells were excluded if the variation in the size of response exceeded ± 10%. Imipramine was studied on 31 cells, desipramine on 39 cells. Both potentiation and antagonism of the response to NA could be observed after a brief application (25-100 nA for 20-60 s) of either of the antidepressants.

Potentiation of the response was seen on 49 cells. A response was regarded as potentiated if there was more than 20% increase over the size of the average control response. The degree of potentiation was 51%-138% (inter-quartile range). The potentiated response had a characteristic time course compared to the control response: the peak of the response was usually higher, and the recovery time longer. The latency of the potentiated response could be either shorter or longer than that of the control response. On a few cells, only one response showed potentiation; on the majority of the cells, however, several responses were potentiated, and the control response recovered only after a longer time (up to 90 minutes).

Antagonism of the response was seen on 45 cells. Antagonism appeared as a reduction in the total spike number compared to the control. This reduction in size varied between 20 and 100%.

The occurrence of potentiation and antagonism followed a well-defined pattern. The following patterns could be observed:

(1) *'Early' potentiation.* In this case the first response after the antidepressant showed the greatest degree of potentiation. (The first application of the monoamine after the antidepressant usually followed less than 1.5 min after the application of the antidepressant.) Subsequent responses became gradually smaller until recovery of the control could be seen. Recovery usually occurred 10-20 min after the antidepressant had been applied. Early potentiation was seen on 18 neurones.

(2) *'Late' potentiation.* In this case, potentiation developed gradually, achieving a maximum 10-30 min after the application of the antidepressant. Recovery occurred 30-60 min after the antidepressant had been applied. Late potentiation was seen on 18 cells. Examples of late potentiation are shown in Figures 1 (left hand traces) and 2.

(3) *Antagonism followed by potentiation.* In this case, the first response after the antidepressant was reduced in size. This initial antagonism was later followed by potentiation, and finally by gradual recovery of the response. This pattern of drug-interaction was observed on 31 cells. Antagonism invariably preceded potentiation, the reverse was never seen. An example of this type of drug-interaction is shown in Figure 1 (right hand traces).

(4) *Antagonism only was seen on 14 cells.* In this case, an initial antagonism of the response was followed by recovery. On some cells, a number of studies were conducted and more than one pattern of drug-interaction could be observed (see below).

There was no qualitative difference between the effects of imipramine and desipramine: both antidepressants modified the response according to one of the four patterns.

In an attempt to identify whether the pattern of drug-interaction was related to the dose of antidepressant applied, we compared the effects of two or more doses of the same antidepressant on responses to noradrenaline on the same cells. The doses of antidepressant applied were within the range used in all of the drug-interaction studies. Increasing doses of imipramine were tested on 6 cells, and the effects of increasing doses of desipramine were studied on 12 cells. We have found that a higher dose was required to cause late potentiation than early potentiation and that a higher dose was required to cause antagonism than potentiation only. An example of the effects of two different doses of desipramine on the size of excitatory responses to noradrenaline is shown in Figure 2.

Table 1 Effects of imipramine and desipramine on single cortical neurones

Antidepressant	*Effect (number of cells)*			
	Excitation	*Depression*	*Reduction in spike amplitude*	*No effect*
Imipramine	14	8	3	119
Desipramine	9	13	3	129

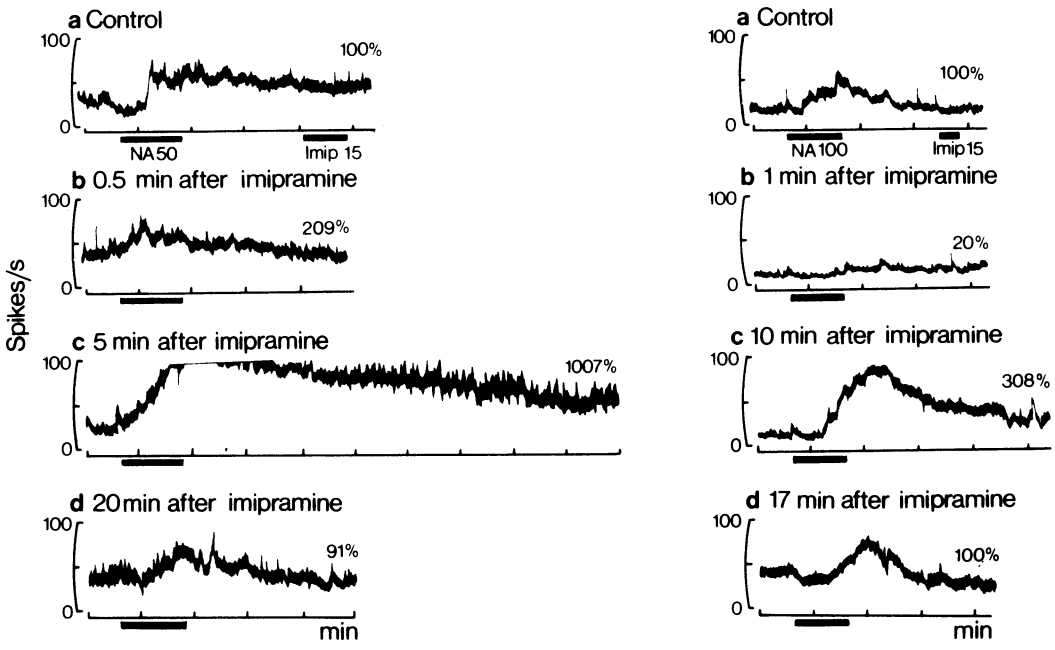

Fig. 1 Excerpts from the ratemeter recording of the firing rate of two cortical neurones. Drug applications are indicated by horizontal bars below the abscissae (numbers refer to intensities of ejecting currents, nA). Figures above the ratemeter trace are the total spike numbers expressed as percentage of the control responses. Left-hand traces, study conducted on one neurone showing 'late' potentiation of excitatory responses to noradrenaline (NA) by imipramine (Imip); right-hand traces, study conducted on another neurone showing antagonism followed by potentiation of excitatory responses to NA by imipramine.

On one neurone excited by noradrenaline imipramine reversed the response into a depression. Later the excitatory response recovered.

Depressant responses. The effects of the two antidepressants were studied on 11 cells depressed by noradrenaline. Imipramine was studied on 6 cells, and desipramine was tested on 8 cells. Both potentiation and antagonism of the depressant responses after a brief application of the antidepressants could be observed (Figure 3). Potentiation was seen on 12 cells, antagonism was observed on 3 cells. The same patterns of drug-interaction were seen as with excitatory responses: (1) early potentiation (3 cells); (2) late potentiation (7 cells); (3) antagonism followed by potentiation (2 cells); (4) antagonism only (1 cell). There was no qualitative difference between the effects of imipramine and desipramine.

On one cell imipramine reversed the depressant response into an excitation (Figure 4).

Effect on responses to 5-hydroxytryptamine

Excitatory responses. Drug-interaction studies were successfully completed on 41 cells excited by 5-HT. Imipramine was studied on 31 cells, and desipramine on 14 cells. Both potentiation and antagonism could be observed after a brief application of either of the antidepressants. Potentiation was observed on 29 cells, antagonism was seen on 31 cells. The same patterns of drug-interaction could be observed as in the case of responses to NA: (1) early potentiation (9 cells); (2) late potentiation (7 cells); (3) antagonism followed by potentiation (13 cells); (4) antagonism alone (18 cells). An example of antagonism followed by potentiation is shown in Figure 5. There was no qualitative difference between the effects of imipramine and desipramine: all four drug-interaction patterns could be observed with either of the antidepressants.

The effects of two or more doses of imipramine

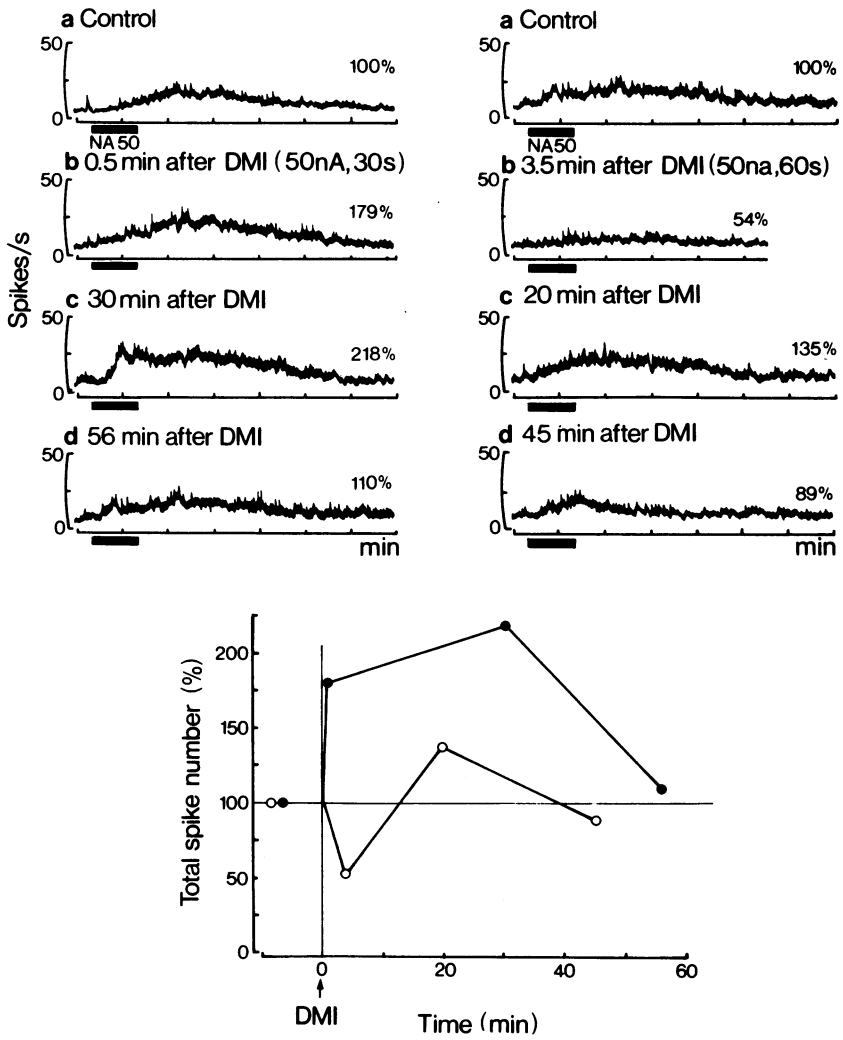

Fig. 2 Excerpts from the ratemeter recording of the firing rate of a single cortical neurone (as in previous Figure). Left-hand traces (•), following a smaller dose of desipramine (DMI) the excitatory response to noradrenaline (NA) was potentiated; right-hand traces (○), following a larger dose of DMI the response was first antagonized, and later potentiated. The graph at the bottom summarizes the time-course of the two studies. It is apparent that the bigger dose of DMI antagonized the response at a time when a smaller dose potentiated it.

were compared on 5 cells. It was found that a higher dose was required to cause antagonism than potentiation only.

Depressant responses. The effects of the two antidepressants were studied on 15 cells depressed by 5-HT. Imipramine was studied on 8 cells, desipramine was investigated on 9 cells. Both potentiation and antagonism of the depressant responses could be observed. Potentiation was seen on 13 cells, antagonism was observed on 6 cells. An example of the potentiation of depressant responses to 5-HT by desipramine is shown in Figure 6. The same patterns of drug-interaction were seen as with excitatory responses: (1) early potentiation (5 cells); (2) late potentiation (6 cells); (3) antagonism followed by potentiation (2 cells); (4) antagonism only (4 cells).

On one cell imipramine reversed the depressant response into an excitation.

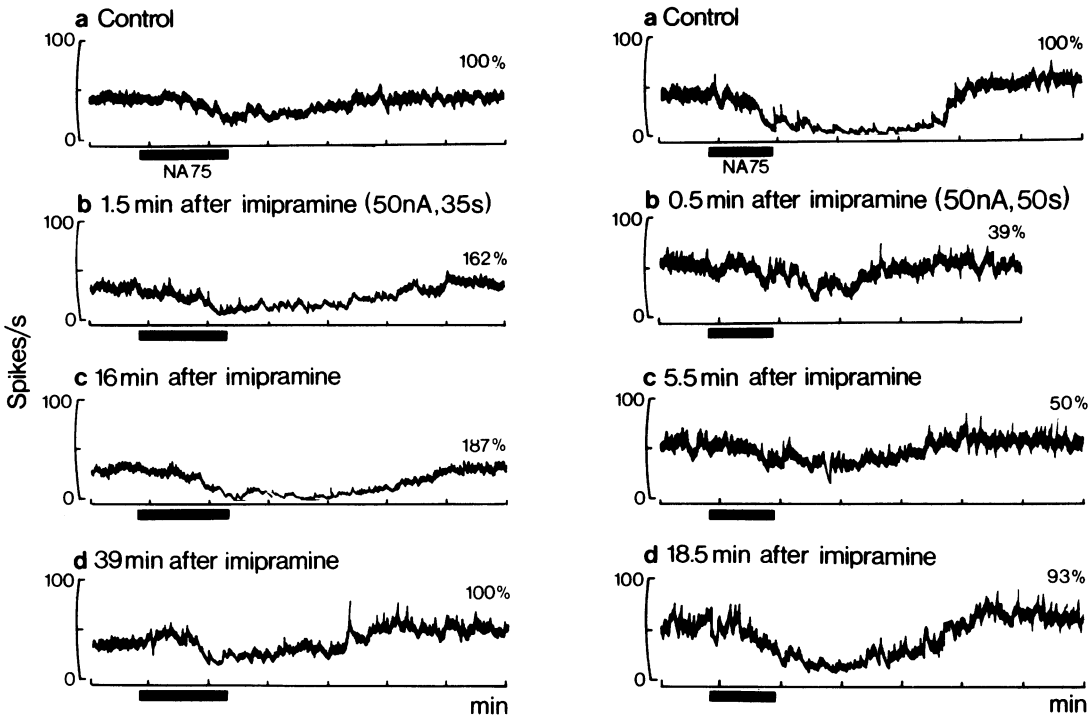

Fig. 3 Excerpts from the ratemeter recording of the firing rate of two cortical neurones (as in previous Figures). Figures above the ratemeter traces refer to the sizes of responses expressed as percentage of the control response (see methods section). Left-hand traces, study conducted on one neurone showing potentiation of depressant responses to noradrenaline (NA) by imipramine; right-hand traces, study conducted on another neurone showing antagonism of depressant responses to NA by imipramine.

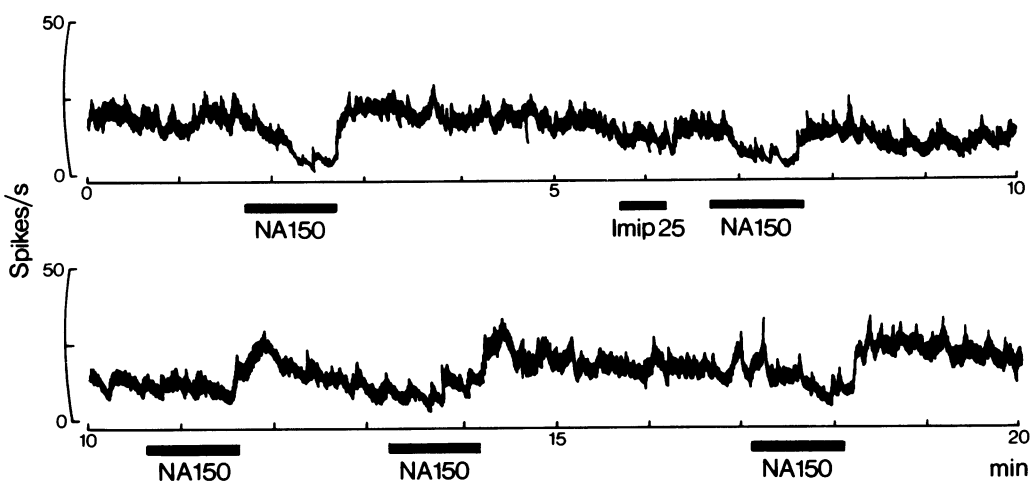

Fig. 4 Continuous ratemeter recording of the firing rate of a single cortical neurone (as in previous Figures). Following a brief application of imipramine (Imip), the depressant response to noradrenaline (NA) was reversed into an excitation; later the depressant response reappeared.

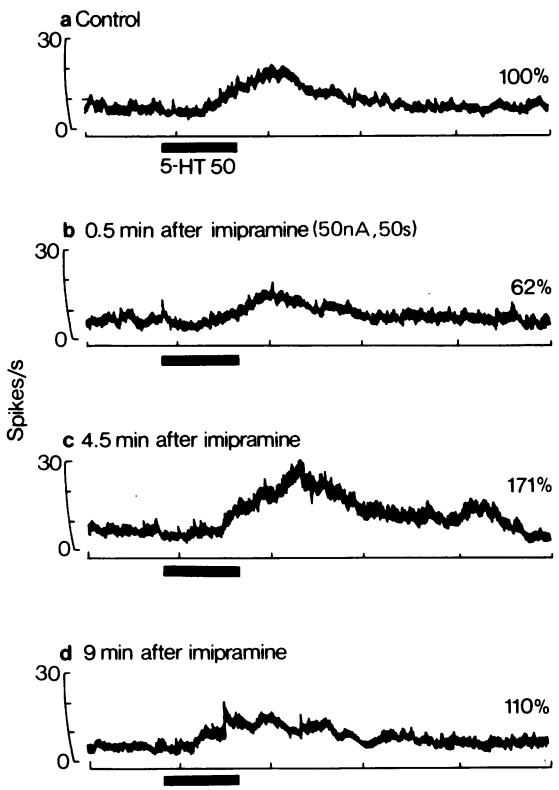

Fig. 5 Excerpts from the ratemeter recording of the firing rate of a single cortical neurone (as in previous Figures). Following a brief application of imipramine, the excitatory response to 5-hydroxytryptamine (5-HT) was first antagonized, and later potentiated.

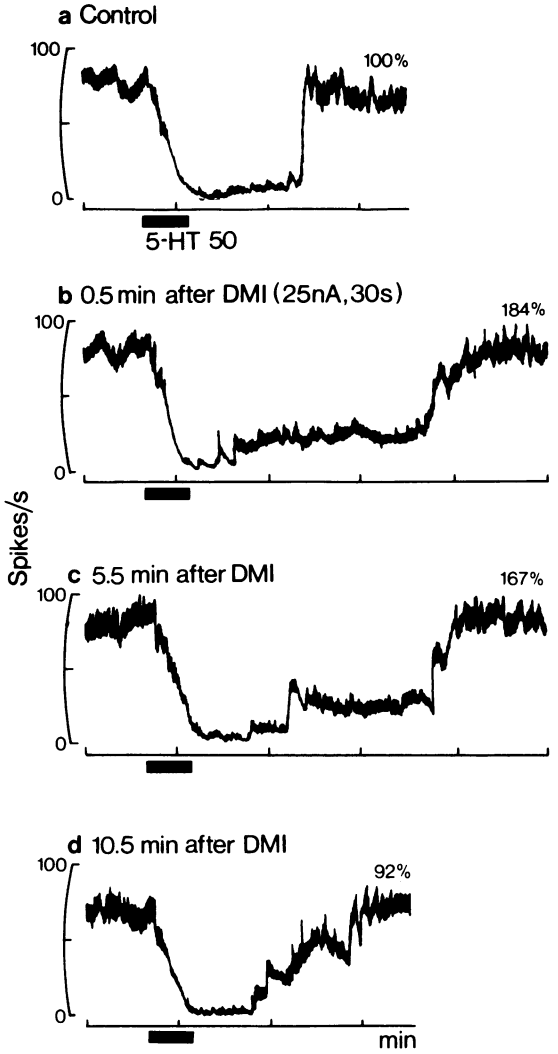

Fig. 6 Excerpts from the ratemeter recording of the firing rate of a single cortical neurone (as in previous Figures). Following a brief application of imipramine, the depressant response to 5-hydroxytryptamine (5-HT) was potentiated.

Effect on responses to glutamate

The effect of desipramine on excitatory responses to glutamate was studied on 9 cells. On most of these cells several different doses of desipramine were tested. The dose of desipramine varied between 10-100 nA applied for 20-300 seconds. The effects of glutamate on the firing rate were tested for 10-20 min following the application of the antidepressant. On none of the cells could any change be observed in the size or time course of responses to glutamate during or after the application of desipramine.

Discussion

In the experiments presented in this paper we used the spontaneously firing cortical neurone as a test system in order to study the interaction between tricyclic antidepressant drugs and the monoamines. These neurones are sensitive to NA and 5-HT applied by microelectrophoresis; the responses to the monoamines are repeatable, and they can be modified by antagonists applied by microelectrophoresis (Roberts & Straughan, 1967; Johnson, Roberts, Sobieszek & Straughan, 1969). Moreover, there is evidence that both NA and 5-HT containing nerve terminals reach the cerebral

cortex (Fuxe, 1965). It is possible, therefore, that the responses to exogenously applied monoamines are mediated by subsynaptic receptors, and that presynaptic processes, such as uptake into monoamine-containing terminals, may influence the responses observed.

A brief application of the antidepressant did not have any effect on the firing rate of the vast majority of cells. On a small number of cells, however, a response to imipramine or desipramine was observed. A possible explanation for this effect of the antidepressants themselves could be that it reflects the interaction between endogenously released monoamine transmitters and the antidepressants. As responses to acetylcholine are also affected by the antidepressants (Bradshaw *et al.*, 1971; Bevan, Bradshaw, Roberts & Szabadi, 1973), an interaction with acetylcholine released by cholinergic inputs to the neurone should also be considered. On a few cells, a reduction in spike amplitude was observed in response to the antidepressant. This probably reflects the local anaesthetic action of these drugs (Domenjoz & Theobald, 1959).

We have found that the tricyclic antidepressants have a dual effect on responses to NA and 5-HT: smaller doses potentiate, and bigger doses antagonize the responses. This dual effect can be interpreted in terms of two independent mechanisms: a more sensitive potentiating mechanism, and a less sensitive antagonizing mechanism. The pattern of occurrence of both potentiation and antagonism in our experiments may be explicable in terms of hypothetical concentration changes following a brief application of the antidepressant (Figure 7). After the brief ejecting pulse, the concentration of the antidepressant probably rises quickly to a peak, and then gradually declines (Castillo & Katz, 1955). When a relatively small dose of antidepressant is applied (Fig. 7, curve 1), the concentration rises fast above the hypothetical threshold for the potentiating mechanism (P), but it does not reach the higher threshold for the antagonizing mechanism (A). Thus potentiation of the response to the monoamine is observed. The first response after the antidepressant, which coincides with the highest antidepressant concentration, will show the greatest degree of potentiation ('early potentiation'). When a slightly bigger dose of antidepressant is applied (Fig. 7, curve 2), the concentration may rise slightly above the antagonism threshold (A). Therefore at the peak of the concentration curve the size of the response to the monoamine will reflect the relationship between the activation of the two mechanisms. Thus a smaller degree of potentiation may be followed by a greater degree of potentiation as the concentration falls below the antagonism threshold ('late potentiation'). If a still bigger dose is applied (Fig. 7, curve 3), the concentration of the antidepressant rises high above the antagonism threshold. Thus the response to the monoamine may be first reduced in size, and potentiation develops later, as the concentration of the antidepressant falls (antagonism followed by potentiation). The occurrence of antagonism followed by recovery (but by no potentiation), does not fit readily into this model. A possible explanation is that the study was not continued for long enough after the appearance of recovery, since this recovery occurred very often after a longer delay (up to 90-100 minutes). Another possibility is that in these cases the existence of a relatively higher concentration of the antidepressant for a longer time results in a persistent binding of the antidepressant to the receptors, thus interfering with the appearance of potentiation when the concentration of the antidepressant falls below the antagonism threshold.

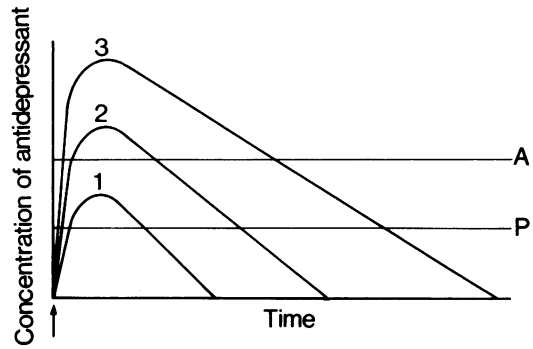

Fig. 7 Hypothetical concentration changes following a brief application of an antidepressant. Horizontal lines show the threshold for the potentiating (P) and antagonizing mechanisms (A) (see text). Curve 1, 2, 3: concentration curves following small, intermediate and large doses of the antidepressant.

The dual effect of the antidepressants on responses to NA and 5-HT may reflect the separate post-synaptic and pre-synaptic actions of the antidepressants. The antagonism of the responses may be due to a post-synaptic receptor blocking action of the antidepressants. There is experimental evidence that imipramine and desipramine block peripheral α-adrenoceptors (Callingham, 1966; Sturman, 1970; McCulloch & Story, 1972), and also have an anti-5-HT activity in peripheral smooth muscle systems (Domenjoz & Theobald, 1959). Potentiation of neuronal responses to the monoamines by imipramine and desipramine may reflect the blockade of uptake of NA and 5-HT

into pre-synaptic terminals. It has been demonstrated that there are powerful uptake mechanisms both for NA and 5-HT in the brain, and that these mechanisms can be blocked by imipramine and desipramine (Glowinski & Axelrod, 1964; Ross & Renyi, 1967; 1969). Furthermore, it has been reported that after pre-treatment with 6-hydroxydopamine, desipramine fails to potentiate responses of Purkinje cells to NA (Hoffer *et al.*, 1971). As 6-hydroxydopamine destroys the NA-containing neurones in the brain (Bloom, Algeri, Groppetti, Revuelta & Costa, 1969), this observation could indicate the importance of monoamine-containing terminals for the potentiation of neuronal responses.

There are, however, observations on single neurones which could not easily be reconciled with the 'uptake blockade hypothesis' of potentiation. We have found that sotalol and methysergide have a dual effect on responses to NA, 5-HT and mescaline: small doses potentiate, and higher doses antagonize the responses (Bevan, Bradshaw & Szabadi, 1974). The potentiation of responses to monoamines by methysergide can hardly be explained by the blockade of uptake, since methysergide is only a very weak blocker of 5-HT uptake (Born, Juengjaroen & Michal, 1972).

As uptake blockade may not be a complete explanation for potentiation, we would like to consider the possibility that not only the antagonism, but also the potentiation of the response is due to post-synaptic effects. There is evidence suggesting that both excitatory and inhibitory receptors to the monoamines can coexist on the same neurone (Szabadi & Bradshaw, 1974). The presence of two kinds of receptor is often apparent in the case of biphasic responses, consisting of a depressant and an excitatory phase. In the case of pure monophasic responses, however, one kind of receptor may be completely masked by the dominant receptors which determine the direction of the observed response. We propose that potentiation may be due to the selective blockade of masked receptors by a smaller concentration of the antidepressant. A higher concentration of the antidepressant would block the dominant receptors as well, thus causing a reduction in the size of the observed response. Figure 1 (left) gives some support to this hypothesis. The response shown in this figure had an initial depressant phase. It is apparent that the appearance of potentiation of the excitatory response was accompanied by the disappearance ('antagonism') of the initial depressant phase, and by a shortening of the response latency. When the original response recovered, the initial depressant phase reappeared. As a smaller dose is required to cause potentiation than antagonism, it would seem that the masked receptors are more sensitive to the antidepressants. On occasions, however, the reverse can be the case: when the dominant receptors are blocked selectively by the antidepressant, the masked receptors would become un-masked, resulting in the reversal of the response (Figure 4). It is interesting that the α-adrenoceptor blocking agent sotalol and the 5-HT receptor blocking agent methysergide have a very similar dual effect on responses to monoamines (Bevan *et al.*, 1974). Moreover, these drugs can also cause reversal of the responses to the monoamines (Szabadi & Bradshaw, 1974). We suggest that all these drugs (imipramine, desipramine, sotalol, methysergide) act as blockers of central monoamine receptors.

Imipramine and desipramine had qualitatively similar effects in our experiments: both drugs affected responses to NA and 5-HT in a similar fashion. In another study, however, we have found that desipramine was more potent than imipramine in modifying responses to NA, whereas the reverse was true in the case of responses to 5-HT (Bradshaw *et al.*, 1973a).

We have found that not only responses to monoamines, but also responses to acetylcholine can be modified by tricyclic antidepressant drugs (Bradshaw *et al.*, 1971; Bevan *et al.*, 1973). Imipramine and desipramine have a dual effect on responses to acetylcholine; smaller doses potentiate, bigger doses antagonize the response. Since atropine has a very similar effect on responses to acetylcholine (Bevan *et al.*, 1973), it is possible that the potentiation of responses to acetylcholine is also due to the blockade of masked receptors. Thus, the tricyclic antidepressants may exert their central actions by blocking central monoamine and acetylcholine receptors.

In conclusion, we can state that imipramine and desipramine can influence responses to NA, 5-HT and acetylcholine in a similar manner. It is possible, however, that a given concentration of the antidepressant has a differential effect on different systems: while the effects of one potential neurotransmitter are potentiated, the effects of another transmitter may be antagonized. Thus the tricyclic antidepressants may change the balance of activity between neuronal systems, and such a change may be the basis of the antidepressant action of these drugs.

This work was supported by the Scottish Home and Health Department and the Mental Health Trust and Research Fund. Imipramine and desipramine were the generous gifts of Geigy (U.K.) Ltd. We are grateful to Mr R. Menzies and Mr R. Lamb for their technical assistance. M.H.T.R. is the Rothschild Fellow of the Schizophrenia Research Fund.

References

AVANZINO, G.L., ERMIRIO, R. & ZUMMO, C. (1971). Effects of microiontophoretic application of imipramine on single neurones in the brain stem. *Neuropharmacology*, **10**, 661-664.

BEVAN, P., BRADSHAW, C.M., ROBERTS, M.H.T. & SZABADI, E. (1973). The dual action of tricyclic antidepressant drugs on responses of single cortical neurones to acetylcholine. *Br. J. Pharmac.*, **49**, 173-174P.

BEVAN, P., BRADSHAW, C.M. & SZABADI, E. (1974). Potentiation and antagonism of neuronal responses to monoamines by methysergide and sotalol. *Br. J. Pharmac.*, **50**, 445P.

BLOOM, F.E., ALGERI, S., GROPPETTI, A., REVUELTA, A. & COSTA, E. (1969). Lesions of central norepinephrine terminals with 6-OH-dopamine: Biochemistry and fine structure. *Science*, **166**, 1284-1286.

BORN, G.V.R., JUENGJAROEN, K. & MICHAL, F. (1972). Relative activities on and uptake by human blood platelets of 5-hydroxytryptamine and several analogues. *Br. J. Pharmac.*, **44**, 117-139.

BRADSHAW, C.M., ROBERTS, M.H.T. & SZABADI, E. (1971). Effect of tricyclic antidepressants on monoamine responses of single cortical neurones. *Br. J. Pharmac.*, **41**, 394-395P.

BRADSHAW, C.M., ROBERTS, M.H.T. & SZABADI, E. (1973a). Comparison of the effects of imipramine and desipramine on single cortical neurones. *Br. J. Pharmac.*, **48**, 358-359P.

BRADSHAW, C.M., ROBERTS, M.H.T. & SZABADI, E. (1973b). Kinetics of the release of noradrenaline from micropipettes: interaction between ejecting and retaining currents. *Br. J. Pharmac.*, **49**, 667-677.

BRADSHAW, C.M. & SZABADI, E. (1972). A technique for achieving greater stability of the brain for microiontophoretic studies of single cortical neurones. *Br. J. Pharmac.*, **45**, 185-186P.

BRADSHAW, C.M., SZABADI, E. & ROBERTS, M.H.T. (1973). The reflection of ejecting and retaining currents in the time-course of neuronal responses to microelectrophoretically applied drugs. *J. Pharm. Pharmac.*, **25**, 513-520.

CALLINGHAM, B.A. (1966). The effects of imipramine and related compounds on the uptake of noradrenaline into sympathetic nerve endings. In: *Antidepressant Drugs, ed. Garattini, S. & Dukes, M.N.G., pp. 35-43. Proc. 1st Int. Symp. Milan, 1966. Amsterdam: Excerpta Medica.*

CASTILLO, J. DEL, & KATZ, B. (1955). On the localisation of acetylcholine receptors. *J. Physiol., Lond.*, **128**, 157-181.

DAVIS, J.M. (1970). Theories of biological etiology of affective disorders. *Int. Rev. Neurobiol.*, **12**, 145-175.

DOMENJOZ, R. & THEOBALD, W. (1959). Zur Pharmakologie des Tofranil (N-(3-dimethylaminopropyl)-iminodibenzyl-Hydrochlorid). *Arch. Int. Pharmacodyn. Thér.*, **120**, 450-489.

FUXE, K. (1965). Evidence for the existence of monoamine neurones in the central nervous system. IV. The distribution of monoamine nerve terminals in the central nervous system. *Acta physiol. scand.*, **64**, suppl. **247**, 39-85.

GLOWINSKI, J. & AXELROD, J. (1964). Inhibition of uptake of tritiated noradrenaline in the intact rat brain by imipramine and structurally related compounds. *Nature, Lond.*, **204**, 1318-1319.

GYERMEK, L. & POSSEMATO, C. (1960). Potentiation of 5-hydroxytryptamine by imipramine. *Med. exp.*, **3**, 225-229.

HERTTING, G., AXELROD, J. & WHITBY, L.G. (1961). Effect of drugs on the uptake and metabolism of ^{3}H-norepinephrine. *J. Pharmac. exp. Ther.*, **134**, 146-153.

HOFFER, B.J., SIGGINS, G.R. & BLOOM, F.E. (1971). Studies on norepinephrine-containing afferents to Purkinje cells of rat cerebellum. II. Sensitivity of Purkinje cells to norepinephrine and related substances administered by microiontophoresis. *Brain Res.*, **25**, 523-534.

IVERSEN, L.L. (1965). Inhibition of noradrenaline uptake by drugs. *J. Pharm. Pharmac.*, **17**, 62-64.

JOHNSON, E.S., ROBERTS, M.H.T., SOBIESZEK, A. & STRAUGHAN, D.W. (1969). Noradrenaline sensitive cells in cat cerebral cortex. *Int. J. Neuropharmac.*, **8**, 549-566.

McCULLOCH, M.W. & STORY, D.F. (1972). Antagonism of noradrenaline and histamine by desipramine in the isolated artery of the rabbit ear. *Br. J. Pharmac.*, **46**, 140-150.

ROBERTS, M.H.T. & STRAUGHAN, D.W. (1967). Excitation and depression of cortical neurones by 5-hydroxytryptamine. *J. Physiol., Lond.*, **193**, 269-294.

ROSS, S.B. & RENYI, A.L. (1967). Inhibition of the uptake of tritiated catecholamines by antidepressant drugs and related agents. *Europ. J. Pharmac.*, **2**, 181-186.

ROSS, S.B. & RENYI, A.L. (1969). Inhibition of the uptake of 5-hydroxytryptamine in brain tissue. *Europ. J. Pharmac.*, **7**, 270-277.

SCHILDKRAUT, J.J. (1965). The catecholamine hypothesis of affective disorders: a review of supporting evidence. *Amer. J. Psychiat.*, **122**, 509-522.

SIGG, E.B., SOFFER, L. & GYERMEK, L. (1963). Influence of imipramine and related psychoactive agents on effects of 5-hydroxytryptamine and catecholamines on cat nictitating membrane. *J. Pharmac. exp. Ther.*, **142**, 13-20.

STURMAN, G. (1970). Modification by a tricyclic series of compounds of the noradrenaline effect on the cat nictitating membrane. *J. Pharm. Pharmac.*, **23**, 142-143.

SZABADI, E. & BRADSHAW, C.M. (1974). The role of physical and biological factors in determining the time-course of neuronal responses. *Neuropharmacology*, **13**, 537-545.

TODRICK, A. & TAIT, A.C. (1969). The inhibition of human platelet 5-hydroxytryptamine uptake by tricyclic antidepressant drugs. The relation between structure and potency. *J. Pharm. Pharmac.*, **21**, 751-762.

(Received April 19, 1974)